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Distribution of enzyme activities within the developing maize (*Zea mays*) kernel in relation to starch, oil and protein accumulation

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The association of enzyme activities in developing kernels with specific storage product accumulation at maturity was analyzed in different parts of *Zea mays* inbred OH43 kernels. Maize kernels were harvested at 20 and 55 days post-pollination and dissected into basal region, pericarp, embryo, lower endosperm, middle endosperm and upper endosperm. Mature (55 days post-pollination) kernel parts were analyzed for starch, total protein, zein and oil content. Immature (20 days post-pollination) kernel parts were assayed for activities of 15 enzymes of sugar and amino acid metabolism. Statistical analyses of the data suggested that glucokinase (EC 2.7.1.2), fructokinase (EC 2.7.1.4) and phosphofructokinase (EC 2.7.1.11) activities were primarily associated with oil accumulation, whereas ADP-glucose pyrophosphorylase (EC 2.7.7.27) and sucrose synthase (EC 2.4.1.13) activities were associated with starch accumulation. The results suggest that oil biosynthesis utilizes invertase-mediated sucrose degradation in a pathway not requiring pyrophosphate, whereas starch biosynthesis utilizes a sucrose synthase-mediated pathway of sucrose degradation in a pathway requiring pyrophosphate. Additional groups of enzyme activities were associated with each other but not with any specific storage product and appeared to be associated with general metabolic activity.

Key words – Maize, oil, protein, pyrophosphate, starch, sucrose metabolism, *Zea mays*.

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Introduction

Developing maize kernels simultaneously accumulate starch, protein and oil (Ingle et al. 1965). The different parts of the kernel accumulate different storage products. The endosperm accumulates primarily starch, whereas the embryo accumulates high concentrations of oil (Earle et al. 1946, Inglett 1970). The primary carbon source for both oil and starch biosynthesis in the developing maize kernel is sucrose. Pulse-chase studies have suggested that about 90% of the carbon arriving to developing kernels is in the form of sucrose, the remainder being composed of organic and amino acids (Moutot et al. 1986). Nitrogen arriving to the kernel is largely in the form of the amino acids serine, aspartate, glutamate and glutamine (Moutot et al. 1986).

It might be expected that the different parts of the developing kernel would differ in their enzyme composition, reflecting the differences in storage product accumulation. By evaluating what enzymes are found in what tissues in relation to the storage products associated with them, the relative importance of a particular enzyme for a particular end-product may be deduced. Previous studies from this laboratory have indicated that there are considerable differences in the enzymic composition of the different parts of the kernel with regard to sucrose and hexose metabolism (Doehlert et al. 1988).

The two enzymes that degrade sucrose in developing kernels are invertase and sucrose synthase. Invertase hydrolyzes sucrose to glucose and fructose. It consists of both soluble and cell-wall-bound forms, all of which are found exclusively in the basal regions of the kernel (Doehlert and Felker 1987, Doehlert et al. 1988). Be-

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cause of its largely apoplastic localization, and because activity was associated with regions of the kernel involved in phloem unloading and subsequent uptake into the endosperm, it was hypothesized that invertase might function to facilitate sucrose unloading by maintaining a sucrose gradient beteen the phloem and the apoplast (Doehlert and Felker 1987). Regardless of its function, invertase activity in the basal region of the kernel results in high concentrations of glucose and fructose in that region (Shannon 1972, Hanft and Jones 1986).

Sucrose synthase catalyzes the reaction whereby sucrose and UDP are converted into fructose and uridine diphosphate glucose (UDP-Glc). In contrast to invertase activity, sucrose synthase activity is found primarily in the endosperm (Doehlert et al. 1988). The importance of sucrose synthase in sucrose degradation in the developing maize kernel is suggested by the shrunken-1 mutant of maize. This mutant is deficient in the major form of sucrose synthase in maize endosperm and produces only 70% of the starch of normal kernels (Chourey and Nelson 1976). That starch is produced by shrunken-1 kernels indicates that invertase and the second form of sucrose synthase can partially compensate for the deficiency in shrunken-1.

Because invertase and sucrose synthase produce different reaction products, different enzymes are required for the subsequent metabolism of their products. Invertase requires hexose kinase activities for the conversion of hexoses into hexose-phosphates. Hexose kinase activities have been found to be much lower than other enzymes of sugar metabolism in developing maize kernels (Doehlert 1987, Doehlert et al. 1988), and it has been previously hypothesized that this activity may limit sucrose utilization in developing kernels. A recent study from this laboratory separated four hexose kinases from developing maize kernels, two of which were specific for glucose and two of which were specific for fructose (Doehlert 1989). Fructose may also be converted into sorbitol by the enzyme ketose reductase (sorbitol dehydrogenase; Doehlert 1987).

In addition to fructokinase activity, sucrose synthase also requires UDP-Glc pyrophosphorylase activity and pyrophosphate (PPi) to convert its products of sucrose degradation into hexose-phosphates. Although UDP-Glc pyrophosphorylase activity is very high in maize kernels (Doehlert et al. 1988), the source of PPi for the reaction is not clear. Possible sources of PPi in developing corn kernels are the biosynthesis of starch, protein, RNA and DNA, all of which produce PPi (Taiz 1986). In contrast, the biosynthesis of oil does not produce PPi. If biosynthesis is the source of PPi for sucrose degradation, one might expect to find sucrose synthase activity associated with storage products producing PPi during their biosynthesis. Data suggesting this hypothesis were observed in a previous study (Doehlert et al. 1988), but product accumulation was not measured in kernel parts at maturity.

In the present study, enzymes of sugar and amino acid metabolism were measured in different parts of OH43 kernels harvested 20 days post-pollination (DPP), and enzyme activities were compared to starch, protein and oil contents in the corresponding parts of 55 DPP kernels.

Abbreviations – BICINE, N,N-bis(2-hydroxyethyl)glycine; DPP, days post-pollination; Fru, fructose; Glc, glucose; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; P-, -phospho-; PPi, pyrophosphate.

Materials and methods

Plant material

Maize (*Zea mays* L. inbred OH43) plants were grown in the field in Peoria, IL, USA, in the summer of 1988. Ears were pollinated by hand and harvested at 20 DPP or 55 DPP. Kernels harvested at 20 DPP were dissected immediately after harvest into the base (lower 2 mm), pericarp, embryo, lower endosperm, middle ensosperm and upper endosperm. These parts were frozen immediately on dry ice, lyophilized, powdered and stored at −80°C until extraction. About 40 to 50 kernels were dissected from each ear for each sample. Kernels harvested at 55 DPP were initially frozen at −80°C and subsequently thawed one kernel at a time and dissected into the same 6 parts described above. These parts were also refrozen, lyophilized, ground to a powder and then stored until analyses were to be performed.

Enzyme assays

Enzymes were extracted by the procedure described earlier (Doehlert et al. 1988). The activities of sucrose synthase (UDP-Glc: D-Fru 2-glucosyltransferase, EC 2.4.1.13), fructokinase (ATP: D-Fru 6-P-transferase, EC 2.7.1.4), glucokinase (ATP: D-Glc 6-P-transferase, EC 2.7.1.2), phosphofructokinase (ATP: D-Fru-6-P 1-P-transferase, EC 2.7.1.11), PPi: Fru-6-P 1-P-transferase (EC 2.7.1.90), aldolase (D-Fru-1,6-bis-P: D-glyceraldehyde-3-P lyase, EC 4.1.2.13), phosphoglucoisomerase (D-Glc-6-P ketoisomerase, EC 5.3.1.9), phosphoglucomutase (D-Glc-1,6-bis-P: D-Glc-1-P P-transferase, EC 2.7.5.1), UDP-Glc pyrophosphorylase (UTP: D-Glc-1-P uridyltransferase, EC 2.7.7.9) and sorbitol dehydrogenase (sorbitol: NAD 5-oxidoreductase, EC 1.1.1.14) were assayed using procedures described earlier (Doehlert et al. 1988).

ADP-Glc pyrophosphorylase (ATP: D-Glc-1-P adenyltransferase, EC 2.7.7.27) activity was measured in undialyzed extracts because of the extreme lability of the activity. Activity was measured in the direction of ADP-Glc degradation by coupling Glc-1-P production with phosphoglucomutase, Glc-6-P dehydrogenase and NAD reduction in a procedure similar to that used by Plaxton and Preiss (1987). Assays contained 50 m*M* HEPES-NaOH (pH 7.2), 5 m*M* $MgCl_2$, 1 m*M* ADP-Glc, 10 m*M* 3-P-glycerate, 1 m*M* NAD, 5 μ*M* Glc-1,6-

bis P, 1 unit phosphoglucomutase, 2.5 units Glc-6-P dehydrogenase and 25 µl of enzyme extract in a total volume of 1 ml. Assays were initiated with 2 m*M* PPi. Activity was derived from the increase in A_{340} as NAD was reduced.

All other assays were also continuous spectrophotometric assays coupled with the oxidation of NADH. The P-glycerate kinase (ATP: 3-P-D-glycerate 1-P-transferase, EC 2.7.2.3) assay contained 50 m*M* HEPES-NaOH (pH 7.2), 5 m*M* $MgCl_2$, 6 m*M* ATP, 0.2 m*M* NADH, 6 units glyceraldehyde-3-P dehydrogenase, 5 units triose-P isomerase and 25 µl enzyme extract in a final volume of 1 ml. Assays were initiated with 5 m*M* 3-P-glycerate. The P-glycerate mutase (2,3-bis P-glycerate: 2-P-D-glycerate phosphotransferase, EC 2.7.5.3) assay contained 50 m*M* HEPES-NaOH (pH 7.2), 5 m*M* $MgCl_2$, 0.2 m*M* NADH, 3 m*M* ADP, 2 units enolase, 5 units pyruvate kinase, 5 units lactate dehydrogenase and 25 µl enzyme extract in a final volume of 1 ml. Assays were initiated with 5 m*M* 3-P-glycerate. Enolase (2-P-glycerate hydrolase, EC 4.2.1.11) assays contained 50 m*M* BICINE-NaOH (pH 8.5), 5 m*M* $MgCl_2$, 3 m*M* ADP, 0.2 m*M* NADH, 5 units pyruvate kinase, 5 units lactate dehydrogenase and 25 µl enzyme extract in a final volume of 1 ml. Assays were initiated with 2 m*M* 2-P-glycerate. An additional blank in which water was substituted for the enzyme extract was also utilized for enolase assays. L-Alanine transaminase (L-alanine:oxoglutarate aminotransferase, EC 2.6.1.2) assays contained 50 m*M* HEPES-NaOH (pH 7.2), 5 m*M* $MgCl_2$, 20 m*M* alanine, 0.2 m*M* NADH, 2.5 units lactate dehydrogenase and 2.5 µl enzyme extract in a final volume of 1 ml. Reactions were initiated with 10 m*M* α-ketoglutaric acid (neutralized). L-Aspartate transaminase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1) assays contained 50 m*M* BICINE-NaOH (pH 8.5), 5 m*M* $MgCl_2$, 0.2 m*M* NADH, 30 m*M* L-aspartate, 2.5 units malate dehydrogenase and 25 µl enzyme extract in a final volume of 1 ml. Assays were initiated with 10 m*M* α-ketoglutaric acid. L-Glutamate dehydrogenase [L-glutamate: NAD^+ oxidoreductase (deaminating), EC 1.4.1.2] assays contained 50 m*M* BICINE-NaOH (pH 8.5), 5 m*M* $MgCl_2$, 0.2 m*M* NADH, 10 m*M* α-ketoglutarate and 25 µl enzyme extract in a final volume of 1 ml. Assays were initiated with 125 m*M* NH_4Cl.

All assays were optimized for crude whole kernel extracts. One unit of activity is defined as the activity necessary to produce 1 µmol of product in 1 min. All assays were run at 30°C.

Kernel composition analyses

Kernel part fresh weights, dry weights and percent moisture were determined by dissecting a sample of 10 kernels and weighing the parts before and after oven drying (60°C for 48 h). Starch content was determined by the procedure described earlier (Doehlert et al. 1988). Total N was determined by semi-micro-Kjeldahl (Association of Official Analytical Chemists 1984). Percent N was multiplied by 6.25 to obtain percent protein. Zeins were extracted by suspending 0.2 g dried tissue in 2 ml 70% (w/v) ethanol containing 0.5% (w/v) Na-acetate and 0.2% (w/v) dithiotheitol and shaking for 1 h at 60°C. Supernatants were clarified by centrifugation, removed, and the extraction procedure was repeated. Supernatants were then pooled (total volume 4 ml), dried down and protein was determined as described above. Oil was analyzed by gas-liquid chromatography (Black et al. 1967).

Data analysis

All analyses were performed on 4 separate samples, each derived from kernels from a separate ear. Analysis of variance and correlation analysis were performed by the ABSTAT (Anderson Bell, Parker, CO, USA) computer program. Cluster analysis was performed by the SAS (SAS Institute, Cary, NC, USA) computer program.

Results

Composition of mature kernel parts

Mature maize kernels were harvested 55 DPP and dissected into the base, pericarp, embryo, lower endosperm, middle endosperm and upper endosperm. Kernel parts were then subjected to chemical analysis to determine their starch, total protein, zein protein and oil contents (Tab. 1). Endosperm fractions contained the most starch, averaging about 79% of the dry weight. Embryos contained only 10% starch. But embryos contained nearly 30% of their dry weight as oil, whereas endosperm contained only 0.5 to 1.5% oil. Embryos also contained the highest percent total protein in the kernel, most of that being non-alcohol-soluble. Endosperm tissue contained a lower percent total protein than the embryo, but contained a much higher percent of alcohol-soluble protein.

Enzyme activities

Of the enzyme activities measured, sucrose synthase, sorbitol dehydrogenase, ADP-Glc pyrophosphorylase and P-glycerate kinase activities were found primarily in the endosperm (Tab. 2).

Glucokinase, fructokinase, phosphofructokinase, phosphoglucomutase and enolase activities were found in significantly higher activities in the embryo. Glutamate dehydrogenase activity was found primarily in the basal region of the kernel. Different enzyme activities were distributed differently within the endosperm. Sucrose synthase, ADP-Glc pyrophosphorylase and fructokinase activities were higher in the upper or middle endosperm and lowest in the lower endosperm. Phosphoglucoisomerase, phosphoglucomutase, sorbitol de-

Tab. 1. Distribution of storage products in mature kernels.

Part	mg DW	mg (%)				
		Starch	Total protein	Non-zein protein	Zein protein	Oil
Base	4.3	0.3 (7)	0.48(11)	0.43(10)	0.05(1)	0.08 (1.9)
Pericarp	15.2	0.8 (5)	0.89 (6)	0.65 (4)	0.24(2)	0.18 (1.2)
Embryo	26.8	2.7(10)	5.36(20)	4.61(17)	0.75(3)	8.01(29.9)
Lower endosperm	40.4	31.7(78)	5.80(14)	2.39 (6)	3.41(8)	0.61 (1.5)
Middle endosperm	94.1	75.3(80)	13.50(14)	6.58 (7)	6.94(7)	0.47 (0.5)
Upper endosperm	61.2	48.3(79)	8.95(15)	4.13 (7)	4.82(8)	0.31 (0.5)
Whole kernel	241.2	159.0(66)	35.00(15)	19.10 (8)	15.90(7)	9.66 (4.0)

hydrogenase, PPi: Fru-6-P 1-P-transferase, P-glycerate kinase, P-glycerate mutase, enolase and aspartate transaminase activities were highest in the lower endosperm and lowest in the upper endosperm. Phosphofructokinase, glucokinase and alanine transaminase activities were evenly distributed throughout the endosperm.

Cluster analysis

Cluster analysis groups variables in 3-dimensional space according to similarities in patterns of correlations among the variables. The associations of enzyme activities and storage products were illustrated by this analysis (Fig. 1). Percent starch, percent zein, sucrose synthase activity and ADP-Glc pyrophosphorylase activity clustered together as a group, labeled Cluster #1. Percent oil, percent non-zein protein, phosphofructokinase, fructokinase and glucokinase activities were grouped together and are labeled Cluster #2. Phosphoglucomutase and enolase activities were also associated with variables clustered around percent oil content and are included as a subgrouping of Cluster #2.

Phosphoglucomutase and enolase activities were also associated with percent total protein and PPi: Fru-6-P 1-P-transferase activity, indicated by Cluster #3. Cluster #4 included P-glycerate kinase, aspartate transaminase, alanine transaminase, phosphoglucoisomerase and P-glycerate kinase activities. Sorbitol dehydrogenase activity was associated with Cluster #4 as a subgroup, rather than with Cluster #1. Several enzyme activities in Cluster #3 and Cluster #4 were associated and are indicated as a subgrouping labeled Cluster #5. Glutamate dehydrogenase activity was not associated with any of the clusters.

Correlation analysis

Correlation matrices for variables within each cluster have been tabulated to indicate the statistical relationships of the various variables. In Cluster #1 (Tab. 3), sucrose synthase activity was highly correlated with ADP-Glc pyrophosphorylase activity and percent starch and zein, but ADP-Glc pyrophosphorylase activity was less significantly correlated with percent starch and zein.

All variables in Cluster #2 (Tab. 4) were highly significant in their correlations with each other. Phosphoglucomutase and enolase activities, included as a sub-

Tab. 2. Activities [μmol min^{-1} (g FW)$^{-1}$] of enzymes of sugar and amino acid metabolism in dissected parts of 20 DPP maize kernels. N.S: not significant.

Enzyme	Base	Pericarp	Embryo	Endosperm			LSD (0.05)
				Lower	Middle	Upper	
Sucrose synthase	1.21	1.10	3.11	9.69	18.90	20.60	11.60
Fructokinase	0.05	0.15	0.49	0.01	0.06	0.14	0.09
Glucokinase	0.09	0.15	0.33	0.07	0.06	0.09	0.11
Phosphoglucoisomerase	6.67	2.90	4.69	7.69	5.85	3.53	2.31
Phosphoglucomutase	18.50	10.10	59.40	29.80	25.00	14.20	20.50
Sorbitol dehydrogenase	1.47	0.59	0.15	4.55	2.31	1.20	1.93
ADP-Glc pyrophosphorylase	0.01	0.10	0.10	0.18	1.99	1.11	0.77
PPi: Fru-6-P 1-P-transferase	1.55	0.28	2.64	2.32	1.54	0.50	1.22
Phosphofructokinase	0.90	0.68	1.84	0.68	0.67	0.66	0.64
P-Glycerate kinase	30.70	12.50	29.70	62.40	51.00	23.00	17.70
P-Glycerate mutase	8.67	3.43	7.44	9.48	7.85	2.86	3.64
Enolase	12.80	4.20	21.80	14.60	12.60	7.30	7.00
Glutamate dehydrogenase	2.52	0.63	0.10	0.03	0.04	0.03	0.21
Alanine transaminase	0.34	0.19	0.45	0.70	0.44	0.21	N.S
Aspartate transaminase	18.30	10.40	29.20	41.50	24.10	14.90	17.50

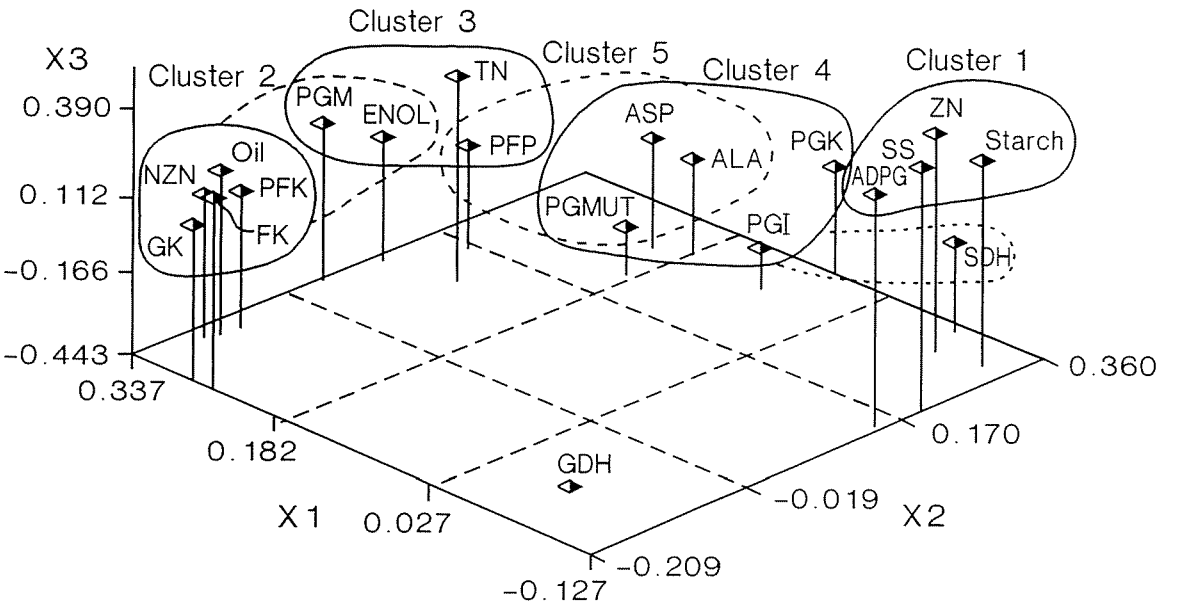

Fig. 1. Cluster analysis of kernel composition and enzyme activities in the different parts of the developing corn kernel. Axes represent eigenvectors in n-dimensional hyperspace, calculated to account for the maximal amount of variation among all variables. Starch, percent starch of dry weight; TN, percent total protein of dry weight; ZN, percent zein (alcohol soluble protein) of dry weight; NZN, percent of non-zein protein (total protein minus zein protein) of dry weight; oil, percent oil of dry weight; ADPG, ADP-Glc pyrophosphorylase activity; SS, sucrose synthase activity; SDH, sorbitol dehydrogenase activity; GK, glucokinase activity; FK, fructokinase activity; PFK, phosphofructokinase activity; PGM, phosphoglucomutase activity; PFP, PPi: Fru-6-P 1-P-transferase activity; ENOL, enolase activity; PGMUT, phosphoglycerate mutase activity; PGI, phosphoglucoisomerase activity; PGK, phosphoglycerate kinase activity; ASP, aspartate transaminase activity; ALA, alanine transaminase activity; GDH, glutamate dehydrogenase activity.

grouping of Cluster #2, were less correlated with the other variables in Cluster #2.

All variables in Cluster #3 were correlated with each other (Tab. 5). All variables in Cluster #4 were also correlated with each other (Tab. 6). Sorbitol dehydrogenase activity was correlated with some variables in Clus-

Tab. 3. Correlation matrix of variables associated with Cluster #1. Values represent correlation coefficients of the corresponding variables in the table. Values followed by a single asterisk (*) are significantly correlated, $P < 0.05$. Values followed by a double asterisk (**) are significantly correlated, $P < 0.01$.

Variable	Sucrose synthase	ADP-Glc pyrophosphorylase	% Starch
ADP-Glc pyrophosphorylase	0.87*	1.00	
Starch	0.91**	0.71	1.00
Zein	0.87**	0.62	0.98**

Tab. 4. Correlation matrix of variables associated with Cluster #2. Values represent correlation coefficients of the corresponding variables in the table. Values followed by a single asterisk (*) are significantly correlated, $P < 0.05$. Values followed by a double asterisk (**) are significantly correlated, $P < 0.01$.

Variables	Fructo-kinase	Gluco-kinase	Phospho-fructokinase	Non-zein protein	% Oil	Phospho-glucomutase
Glucokinase	0.97**	1.00				
Phosphofructokinase	0.91**	0.94**	1.00			
Non-zein protein	0.81*	0.82*	0.97**	1.00		
% Oil	0.94**	0.96**	0.99**	0.92**	1.00	
Phosphoglucomutase	0.75	0.77*	0.89**	0.86*	0.92**	1.00
Enolase	0.53	0.56	0.79*	0.84*	0.78*	0.94**

Tab. 5. Correlation matrix of variables associated with Cluster #3. Values represent correlation coefficients of the two corresponding variables in the table. Values followed by a single asterisk (*) represent a significant correlation, $P < 0.05$. Values followed by a double asterisk (**) represent a highly significant correlation, $P < 0.01$.

Variable	Phospho-glucomutase	PPi:Fru-6-P 1-P-transferase	Enolase
PPi:Fru-6-P 1-P-transferase	0.86*	1.00	
Enolase	0.94**	0.96**	1.00
Total Protein	0.84*	0.75	0.85*

ter #4 and is included as a subgroup. Sorbitol dehydrogenase was not correlated with any variable in Cluster #1. All variables within Cluster #5, which included variables from Clusters #3 and #4, were correlated with each other (Tab. 7).

Discussion

The association of distinct groups of enzyme activities involved in sucrose degradation with specific storage products suggests that the pathway of sucrose degradation in the various parts of the corn kernel may be dependent on the metabolic destination of that carbohydrate. Sucrose degradation in maize endosperm appears to be mediated by sucrose synthase and results primarily in starch biosynthesis (Fig. 1, Tab. 3). Sucrose degradation by sucrose synthase requires PPi for the pyrophosphorylysis of UDP-Glc. No other enzyme activity can be identified that can convert UDP-Glc into glucose-phosphates in developing maize kernels (Doehlert et al. 1988). PPi is produced during starch biosynthesis by ADP-Glc pyrophosphorylase. Starch accumulation, sucrose synthase activity and ADP-Glc pyrophosphorylase activity were all correlated with each other (Tab. 3). The association of sucrose synthase activity with starch biosynthesis has been noted by several other investigators in other tissues (Murata et al. 1966, de Fekete 1969, Pressey 1969, Claussen et al. 1986).

Oil accumulation was associated with enzymes involved in the PPi-independent sucrose degradation, such as fructokinase, glucokinase and phosphofructokinase (Fig. 1, Tab. 4). Although invertase activity is not found in developing maize embryos (Doehlert and Felker 1987, Doehlert et al. 1988), high invertase activity is found in the basal endosperm adjacent to the scutellum, and the embryo is in contact with high concentrations of hexoses (Shannon 1972, Hanft and Jones 1986). This invertase activity is distinctly separated from most of the kernel sucrose synthase activity, which is primarily located in the upper and middle endosperm (Doehlert and Felker 1987, Doehlert et al. 1988). The embryo may primarily utilize apoplastic hexoses derived from invertase activity to make oil. High activities of the hexose kinases and phosphofructokinases would allow for sugar metabolism independent of PPi during oil biosynthesis.

Huber and Akazawa (1986) introduced the concept of the existence of two distinct pathways for sucrose degradation, one mediated by sucrose synthase and dependent on PPi and one mediated by invertase and independent of PPi, but the functional relationship of these pathways was not clear. The data presented here suggest that sucrose to be utilized for oil biosynthesis is broken down by invertase, and metabolism proceeds by a pathway independent of PPi. Sucrose to be utilized for starch biosynthesis is metabolized by sucrose synthase

Tab 6. Correlation matrix of variables associated with Cluster #4. Values represent correlation coefficients of the two corresponding variables in the table. Values followed by a single asterisk (*) indicate a significant correlation, $P < 0.05$. Values followed by a double asterisk (**) represent a highly significant correlation, $P < 0.01$.

Variables	Phosphogluco-isomerase	P-Glycerate kinase	P-Glycerate mutase	Alanine transaminase	Aspartate transaminase
P-Glycerate kinase	0.86*	1.00			
P-Glycerate mutase	0.94**	0.78*	1.00		
Alanine transaminase	0.84*	0.91**	0.84	1.00	
Aspartate transaminase	0.76*	0.85*	0.77*	0.98**	1.00
Sorbitol dehydrogenase	0.78*	0.88**	0.57	0.77*	0.71

Tab. 7. Correlation matrix of variables associated with Cluster #5. Values represent correlation coefficients of the two corresponding variables. Values followed by a single asterisk (*) represent a significant relationship, $P < 0.05$. Values followed by a double asterisk (**) represent a highly significant relationship, $P < 0.01$.

Variables	PPi:Fru-6-P 1-P-transferase	P-Glycerate kinase	Alanine transaminase
P-Glycerate kinase	0.84*	1.00	
Alanine transaminase	0.84*	0.84*	1.00
Aspartate transaminase	0.86*	0.77*	0.98**

in a pathway requiring PPi. Since mature endosperm is composed of 80% starch, it appears likely that the PPi required for sucrose degradation could be largely derived from starch biosynthesis. The dependence of sucrose degradation on PPi generated by biosynthesis would provide a regulatory mechanism linking the degradation of sucrose with biosynthesis. Sucrose would not be degraded by sucrose synthase until sufficient PPi was generated by biosynthesis. The regulation of invertase activity is not understood.

A critical evaluation of the proposed roles of invertase and sucrose synthase in carbon partitioning and the central role of PPi in starch biosynthesis generates two major points of uncertainty. These are: 1) the role of alkaline pyrophosphatase in starch biosynthesis and 2) the relative importance of the pyruvate dehydrogenase complex vs acetyl-CoA synthetase in the generation of acetyl-CoA in oil biosynthesis.

The presence of alkaline pyrophosphatase in plastids, including non-green plastids, has been well demonstrated (Simmons and Butler 1969, Gross and ap Rees 1986, Weiner et al. 1987). It has been suggested by several investigators that this pyrophosphatase functions to hydrolyze PPi generated during starch synthesis, which is in contradiction to the role proposed here of PPi in regulating sucrose degradation. However, the plastid alkaline pyrophosphatase may be linked to a proton pump, as suggested by Rubtsov et al. (1976) in a way analogous to chromatophores of *Rhodospirillum rubrum* (Shakhov et al. 1982), maize mitochondria (Kowalczyk and Maslowski 1981) or maize coleoptile tonoplasts (Chanson et al. 1985), among other systems. Such a system could shift equilibria to favor pyrophosphate formation. The observation that chloroplast pyrophosphatase is a membrane-bound enzyme (Simmons and Butler 1969) supports this hypothesis. Clearly the details of PPi metabolism must be worked out before conclusions can be reached as to the source of PPi during metabolism.

For this analysis it has been assumed that acetyl-CoA synthesis in the developing kernel proceeds via the pyruvate dehydrogenase complex in the plastid. However, an additional mechanism for the generation of acetyl-CoA has been demonstrated in photosynthetic tissue involving acetyl-CoA synthetase (Stumpf 1980, 1987). It was found that acetyl-CoA produced in the mitochondria may be hydrolyzed to yield acetate which is taken up by the plastid. The chloroplast may then generate acetyl-CoA from acetate, coenzyme-A and ATP, producing acetyl-CoA, AMP and PPi. Obviously, if this mechanism is occurring in developing maize embryos, then the association of PPi-independent sucrose degradation enzymes with oil-rich tissue cannot be caused by the lack of PPi generation in plastids. The relative importance of these two pathways for oil synthesis in developing maize embryos is not understood. The presence of substantial PPi: Fru-6-P 1-P-transferase, sucrose synthase (Tab. 2) and UDP-Glc pyrophosphorylase activities (Doehlert et al. 1988) and the accumulation of non-zein proteins (Tab. 1) in the embryo indicate that PPi-dependent metabolism does occur in this tissue. Nevertheless, the association of glucokinase, fructokinase and phosphofructokinase activities with oil accumulation clearly suggests a functional relationship between the PPi-independent pathway of sucrose metabolism and oil accumulation.

Distinct associations are also apparent in the partitioning of proteins in the developing maize kernel (Fig. 1). Zeins are almost exclusively stored in the endosperm, and their levels are highly correlated with enzymes associated with starch biosynthesis (Fig. 1, Tab. 3). The storage proteins in embryos are largely globulins (Khavkin et al. 1978), thus the association of non-zein proteins with oil in the embryo (Fig. 1, Tab. 4) may be due in part to the accumulation of globulins.

Additional clusters of enzyme activities (Fig. 1, Tabs 5–7) appear to be associated with general metabolic activity and not necessarily associated with the deposition of any particular storage product. Many of these enzymes are found primarily in the lower endosperm (Tab. 1), which does not appear to be accumulating very much starch at 20 DPP (data not shown). The presence of high activities of many enzymes in the lower endosperm (Tab. 1) suggests that there is considerable metabolic activity in this region of the kernel, but the function of that activity is not indicated by this study.

Glutamate dehydrogenase is unusual compared with the other enzymes examined in this study in that it is almost entirely found in the basal region of the kernel. This is consistent with the observations of Raczynska-Bojanowska et al. (1986). Another enzyme of glutamate metabolism, glutamine synthetase, is also found largely in the basal (or pedicel) region (Lyznik et al. 1985, Raczynska-Bojanowska et al. 1986, Muhitch 1988). It has been hypothesized that the pedicel region of developing maize kernels may be specialized in glutamate metabolism and amino acid interconversions (Lyznik et al. 1985, Raczynska-Bojanowska et al. 1986, Muhitch 1988).

Hexose kinase activities were lower than all other enzyme activities measured (Tab. 2). The correlation of hexose activities with oil content suggests that hexose kinase activities, along with phosphofructokinase activity, may be limiting sugar utilization for oil biosynthesis. The correlation of sucrose synthase activity with starch accumulation suggests that this enzyme activity may limit sugar utilization for starch biosynthesis. Although the total catalytic capacity of sucrose synthase is much higher than the hexose kinases in endosperm (Tab. 2), the affinities of the hexose kinases for sugars [fructokinase K_m(fructose) = 0.13 m*M*, glucokinase K_m(glucose) = 0.1 to 0.8 m*M*; Doehlert 1989] are much higher than that of sucrose synthase [K_m(sucrose) = 40 m*M*; Tsai 1974]. Thus, at low sugar concentrations, sucrose synthase activity could limit sugar utilization.

In summary, the activities of sucrose synthase and

ADP-Glc pyrophosphorylase have been found to be associated with starch biosynthesis, whereas the activities of phosphofructokinase, fructokinase and glucokinase have been found to be associated with oil biosynthesis in developing corn kernels. It is suggested that starch biosynthesis proceeds from sucrose metabolized by sucrose synthase in a pathway requiring PPi, whereas oil biosynthesis proceeds from sucrose hydrolyzed by invertase in a pathway independent of PPi.

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